STIC-ILL

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10/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

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IMMUNOHISTOCHEMICAL AND PHARMACOKINETIC CHARACTERIZATION OF THE SITE-SPECIFIC IMMUNOCONJUGATE CYT-356 DERIVED FROM ANTIPROSTATE MONOCLONAL ANTIBODY 7E11-C5
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ABSTRACT: In this study, a site-specific immunoconjugate, designated CYT-356, of the prostate-reactive monoclonal antibody 7E11-C5 was characterized by immunohistological methods for reactivity with normal and neoplastic human tissues. In addition, CYT-356 labeled with 111In was assessed by in vivo imaging and pharmacokinetic studies for localization to human tumor xenografts in nude mice. The native antibody and the site-specific immunoconjugate exhibited similar patterns of reactivity with normal human tissues. Although the majority of tissues tested were negative, weak reactivity with cardiac muscle, proximal kidney tubules, and sweat glands was observed. Positive staining of normal prostate epithelial cells and glandular lumina and strong reactivity with a subset of skeletal muscle cells were also observed. CYT-356 reacted with 100% of prostate tumors examined but was negative on a variety of other neoplasms. Following i.v. administration, CYT-356-111In rapidly localized to and imaged LNCaP human prostate adenocarcinoma xenografts in nude mice, reaching maximal levels of about 30% of injected dose/g of tumor within 3 days. No unusual localization was seen to any nontumor tissue or organ; the level of radioactivity in the normal tissues and organs was at or below that seen in the blood. The localization to xenografts was antigen specific and the accessible binding sites in 100-200-mg tumors appeared to be saturated at an antibody dose between 10 and 100 .mu.g. These findings suggest that the CYT-356 immunoconjugate may be useful in the diagnosis and therapy of prostate cancer.

10/7/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

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Immunoscintigraphy of prostatic cancer: Preliminary results with sup 1sup 1sup 1In-labeled monoclonal antibody 7E11-C5.3 (CYT-356)
Wynant G.E.; Murphy G.P.; Horoszewicz J.S.; Neal C.E.; Collier B.D.; Mitchell E.; Purnell G.; Tyson I.; Heal A.; Abdel-Nabi H.; Winzelberg G.
CYTOGEN Corporation, 600 College Road East, Princeton, NJ 08540 United States
Prostate (PROSTATE) (United States) 1991, 18/3 (229-241)
CODEN: PRSTD ISSN: 0270-4137
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LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

A phase 1 study was conducted with the investigational immunoscintigraphic agent, sup 1sup 1sup 1In-CYT-356, a radiolabeled, site-specific immunoconjugate of monoclonal antibody 7E11-C5.3, in 40

Stable clones of murine hybridomas 7E11-C5 and 9H10-A4 were obtained following immunization with LNCaP cells. The LNCaP cells were isolated from a human prostatic cancer (Ca). Both hybridomas secreted monoclonal antibodies (MoAb) of the IgGI subclass which were reactive with the insoluble, cytoplasmic, membrane rich fractions of the immunogen. Neither MoAb reacted with the soluble cytosol of LNCaP cells nor with purified human prostatic acid phosphatase (PAP) nor prostate specific antigen (PSA). MoAb 9H10-A4 reactivity was very narrow and limited to the surfaces of LNCaP cells only. MoAb 7E11-C5 specificity was restricted to human prostatic epithelium, both normal and malignant. Except LNCaP, none of the 32 lines of human normal or neoplastic cells reacted with MoAb 7E11-C5. In a survey of frozen sections from 175 humans specimens, positive indirect immunoperoxidase staining was limited to epithelium in all 11 specimens of localized and metastatic CaP, 7 benign prostatic hypertrophy (BPH) cases and 7 normal prostates. None of the 26 various nonprostatic tumors nor 120 out of 122 specimens from 28 different normal organs were reactive. Positive staining occurred in 2 out of 14 normal kidneys. Competitive binding with MoAb 7E11-C5 or its F(ab')inf 2 fragments demonstrated the presence of circulating epitope 7E11-C5 in 20 out of 43 sera from CaP patients. Only 3 out of 66 sera from nonprostatic malignancies reacted. None of 30 normal blood donors sera nor 7 BPH sera were positive. Thus, highly significant ($p<0.0001$) association between diagnosed prostatic cancer and circulating molecules expressing the epitope reactive with MoAb 7E11-C5 was established. Significant probability ($p<0.05$) also suggested that patients with positive ELISA test are more likely to be in progression, than those who are negative. These results suggest that this apparently new antigenic marker may be of clinical potential in CaP.

Philly Gimbel 1644
6/24
prostate

10/7/9 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06681043 90381728 PMID: 1698122
Immunohistochemical and pharmacokinetic characterization of the site-specific immunoconjugate CYT-356 derived from antiprostate monoclonal antibody 7E11-C5.
Lopes A D; Davis W L; Rosenstraus M J; Uveges A J; Gilman S C
Department of Biological Research, Cytogen Corporation, Princeton, New Jersey 08540.
Cancer research (UNITED STATES) Oct 1 1990, 50 (19) p6423-9, ISSN 0008-5472 Journal Code: 2984705R
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[CANCER RESEARCH 50, 6423–6429, October 1, 1990]

Immunohistochemical and Pharmacokinetic Characterization of the Site-specific Immunoconjugate CYT-356 Derived from Antiprostate Monoclonal Antibody 7E11-C5

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ABSTRACT

In this study, a site-specific immunoconjugate, designated CYT-356, of the prostate-reactive monoclonal antibody 7E11-C5 was characterized by immunohistological methods for reactivity with normal and neoplastic human tissues. In addition, CYT-356 labeled with ^{111}In was assessed by *in vivo* imaging and pharmacokinetic studies for localization to human tumor xenografts in nude mice. The native antibody and the site-specific immunoconjugate exhibited similar patterns of reactivity with normal human tissues. Although the majority of tissues tested were negative, weak reactivity with cardiac muscle, proximal kidney tubules, and sweat glands was observed. Positive staining of normal prostate epithelial cells and glandular lumina and strong reactivity with a subset of skeletal muscle cells were also observed. CYT-356 reacted with 100% of prostate tumors examined but was negative on a variety of other neoplasms. Following i.v. administration, CYT-356-^{111}In rapidly localized to and imaged LNCaP human prostate adenocarcinoma xenografts in nude mice, reaching maximal levels of about 30% of injected dose/g of tumor within 3 days. No unusual localization was seen to any nontumor tissue or organ; the level of radioactivity in the normal tissues and organs was at or below that seen in the blood. The localization to xenografts was antigen specific and the accessible binding sites in 100–200-mg tumors appeared to be saturated at an antibody dose between 10 and 100 μg. These findings suggest that the CYT-356 immunoconjugate may be useful in the diagnosis and therapy of prostate cancer.

INTRODUCTION

Carcinoma of the prostate is the third most frequent cancer and the third leading cause of cancer death in men (1). In the United States in 1988, there were an estimated 99,000 new cases and 28,000 deaths due to prostate cancer. Early detection and treatment are essential for reducing mortality in this disease (2). Tumor-specific or prostate tissue-specific monoclonal antibodies may be useful in this regard (3–6). One such monoclonal antibody is a murine IgG1 designated 7E11-C5, which recognizes a new antigenic marker on prostate epithelial cells (7). The monoclonal antibody is secreted by a hybridoma that resulted from the fusion of the murine myeloma P3-X63Ag8.653 with spleen cells of mice immunized with the human prostate adenocarcinoma cell line LNCaP (8, 9). The antibody reacts weakly with normal and strongly with malignant prostatic epithelium but does not react with nonprostatic tumors or most normal organs. In addition, the antibody is reported to react with a soluble antigen in the serum of patients with prostatic carcinoma (7).

This study was undertaken to determine whether antibody 7E11-C5 could specifically localize to prostatic tumors in a model system. A site-specific GYK-DTPA[2] immunoconjugate of this antibody, designated CYT-356, was characterized by immunohistological methods for reactivity with normal and neoplastic human tissues and by *in vivo* imaging and pharmacokinetic studies for localization to human tumor xenografts in nude mice.

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[2] The abbreviations used are: GYK-DTPA, glycyl-tyrosyl-(N-α-diethylenetriamine pentaacetic acid)-lysine; PBS, phospitate-buffered saline; TXPBS, 0.025% Triton X-100 in phosphate-buffered saline; ABC, avidin-biotinylated horseradish peroxidase complex; ID, injected dose.

MATERIALS AND METHODS

Antibodies and Immunoconjugates. Monoclonal antibody 7E11-C5 was purified from ascites fluid by Protein A affinity chromatography to greater than 90% purity (as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The purified antibody was oxidized to site-specifically generate reactive aldehyde groups on the oligosaccharides found on the heavy chains. GYK-DTPA was coupled to these aldehyde groups as described previously (10, 11). Briefly, antibody at 1 mg/ml in phosphate buffer (0.33 g/liter $Na_2HPO_4 \cdot 7\ H_2O$, 1.22 g/liter $NaH_2PO_4 \cdot H_2O$, 8.8 g/liter NaCl, pH 6.0) was oxidized for 1 h at ambient temperature with 10 mM $NaIO_4$. Following desalting on a Sephadex G-50 column, the oxidized antibody was incubated with a 2000-fold molar excess of GYK-DTPA, in the presence of 10 mM $NaCNBH_3$, overnight at ambient temperature in the dark. The resulting immunoconjugate, CYT-356, was separated from reactants using a TSK-250 size exclusion high performance liquid chromatography column (Bio-Rad, Richmond, CA). Conjugates prepared in this manner retained greater than 80% of their immunoreactivity, as assessed by enzyme-linked immunosorbent assay (data not shown). Similarly, the mouse IgG1 monoclonal antibodies B72.3 (CellTech, Slough, UK) and BL-3 (Damon Biotech, Needham Heights, MA) were purified and site-specifically conjugated to GYK-DTPA as described above. The immunoconjugate B72.3-GYK-DTPA was designated CYT-103.

Radiolabeling Method. Labeling of CYT-356 and CYT-103 with ^{111}In was done on the day of injection. One mCi of $^{111}InCl_3$ (Amersham, England), adjusted to pH 6.0 with 0.5 M sodium acetate buffer, was added to 100 μg of the conjugate and incubated for 1 h at 37°C. Unchelated ^{111}In was removed with a Chelex 100 metal-chelating resin column (Bio-Rad), followed by high performance liquid chromatography on a TSK-G3000SW column (Phenomonex, Ranch Palo Verdes, CA). Eluted fractions containing the IgG monomer peak were pooled and injected into the animals.

Human Tissue and Tumor Specimens. Normal human tissues were obtained from trauma victims within 8 h of death by the Human Tissue Resource Laboratory, directed by Dr. James Resau (University of Maryland). The tissues were placed in cryomolds (American Scientific Products, Edison, NJ), covered with optimal cutting temperature compound (American Scientific Products), immediately frozen in isopentane over liquid nitrogen, and stored at −70°C until used.

In addition, a panel of three to seven frozen specimens of each of 10 different human tumor types and 10 specimens of prostate carcinomas were stained by Impath Laboratories (New York, NY). Tissue diagnosis was made using standard morphological criteria (hematoxylin/eosin-stained sections).

Human Tumor Cell Lines. The LNCaP human prostate tumor cell line was the kind gift of its originator, Dr. Julius Horoszewicz (Buffalo, NY). The DU-145 (12) and PC-3 (13) cell lines were obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in antibiotic-free Eagle's minimum essential medium, supplemented with 10% fetal bovine serum and essential and nonessential amino

Table 1 *Reactivity of native 7E11-C5 and CYT-356 (7E11-C5-GYK-DTPA) with normal human tissues*

Acetone-fixed frozen normal human tissue sections were stained by the ABC method with antibodies at a concentration of 10 μg/ml. Staining with the irrelevant immunoconjugate BL-3-GYK-DTPA was used to define the background.

Tissue	Cell type or structure	Reactivity	
		7E11-C5	CYT-356
Liver	Hepatocytes	–[a]	–
	Portal tract	–	–
Kidney	Glomerulus	–	–
	Proximal tubules	±	±
	Henle's loop	–	–
	Distal tubules	–	–
	Collecting tubules	–	–
Adrenal	Cortex	–	–
	Medulla	–	–
Lymph node		–	–
Heart	Cardiac muscle	±	±
Skin	Epidermis	–	–
	Glands	±	±
	Reticular fibers/nerve	–	–
	Smooth muscle	ND	–
Lung	Alveoli	–	–
	Bronchial epithelium	–	–
	Bronchial glands	–	–
	Muscle	–	–
	Macrophages	–	–
	Chondrocytes	–	–
Esophagus		–	–
Stomach		–	–
Small intestine		–	–
Colon		–	–
Spleen		–	–
Thyroid		–	–
Pancreas	Islets	–	–
	Acinar cells	–	–
Prostate		±	+
Testis		–	–
Skeletal muscle		ND	++
Brain		–	–

[a] Staining was scored as: –, no staining above background; ±, weak positive staining; +, positive staining; ++, strong positive staining; ND, not done or cell type/structure was not seen.

acids, at 37°C in an atmosphere of 5% carbon dioxide in air. For the initiation of tumor growth, cells were trypsinized from monolayer logarithmic phase growing cultures, suspended in medium, centrifuged, resuspended in serum-free medium, counted, centrifuged, and resuspended in serum-free medium or saline at an appropriate density for injection.

Immunoperoxidase Staining. Cryostat-cut frozen sections (10-μm thick) were placed onto precleaned (95% alcohol) microscope slides that had been treated with Histostick (Accurate Chemical and Scientific Corp., Westbury, NY). The tissues were fixed in acetone at 4°C for 10 min and stored at −70°C until used. For staining, slides were allowed to come to room temperature and rehydrated by immersion for 10 min in PBS (7.52 g/liter K_2HPO_4, 1.32 g/liter $NaH_2PO_4 \cdot H_2O$, 7.2 g/liter NaCl, pH 7.2). All operations were done at room temperature unless otherwise noted. Endogenous peroxidase activity was quenched by immersing the slides in 1% H_2O_2 in PBS for 10 min, followed by a 20-min wash in PBS. Nonspecific binding of biotin/avidin reagents was blocked by incubating kidney and liver sections with avidin D blocking solution (Vector Laboratories, Burlingame, CA) for 15 min, rinsing briefly with PBS, incubating with biotin blocking solution for 15 min (Vector Laboratories), and rinsing again in PBS. Following a 20-min incubation with 1% normal horse serum (Vector Laboratories) in PBS to reduce nonspecific binding of the secondary antibody, sections were incubated overnight at 4°C with 7E11-C5 antibody or CYT-356 or BL-3-GYK-DTPA immunoconjugate at 10 μg/ml [diluted in PBS containing 1% bovine serum albumin and 0.1% Thimerosal (Sigma, St. Louis, MO)]. Following a 10-min wash in TXPBS, sections were treated with biotinylated horse anti-mouse antibody (Vectastain anti-mouse ABC kit; Vector Laboratories) for 30 min, followed by a 10-min wash in TXPBS. Sections were then incubated with ABC (Vectastain ABC kit) for 60 min and washed in TXPBS for 10 min, followed by a 10-min wash in 0.25% Triton X-100 in PBS. The slides were then immersed in 3,3'-diaminobenzidine tetrahydrochloride (Sigma) substrate solution (equal volumes of 0.02% H_2O_2 in distilled water and of 0.1% 3,3'-diaminobenzidine tetrahydrochloride, 0.1 M Tris, pH 7.2) for 7 min, followed by a 5-min running tap water rinse. Sections were counterstained with hematoxylin (Sigma), dehydrated in alcohol, cleared in xylene, and mounted with Permount (Fisher Scientific, Fair Lawn, NJ). Sections were examined by visible light microscopy for the presence of dark brown staining.

Mice. Male athymic (*nu/nu*) Swiss background nude mice, 4–6 weeks old, were purchased from Taconic Farms (Germantown, NY), were housed in sterilized cages with filter bonnets, and were given autoclaved laboratory rodent chow (Purina, St. Louis, MO) and filtered tap water *ad libitum*.

Xenograft Tumor Growth. For LNCaP, DU-145, or PC-3 xenograft tumor growth, mice were given s.c. injections in the left rear flank of 1×10^7 cells in exponential growth phase, in about 0.2 ml of sterile medium or saline. In some instances, LNCaP tumors were propagated by s.c. implantation of tumor fragments (~2-mm cubes) aseptically transferred from donor into recipient mice. Recipient mice were given injections of 2.5 mg of cyclophosphamide i.p. 1 day before tumor cells or fragments were injected.

Tumor Localization and Pharmacokinetic Studies. The pharmacokinetics of CYT-356-^{111}In were determined in both LNCaP tumor-bearing and normal nude mice by injection of conjugate i.v. on day 0 into three groups of five mice bearing measurable s.c. LNCaP tumors and into three groups of three mice without tumors. On days 1, 3, and 7, one group of tumor-bearing and one group of normal mice were killed and dissected for biodistribution determinations. Immediately prior to dissection, tumor-bearing mice were imaged using a Starcam II gamma-camera (General Electric, Milwaukee, WI).

The effect of varying the dose of CYT-356-^{111}In on tumor localization was determined in LNCaP tumor-bearing nude mice by injection of 3-, 10-, 100-, or 1000-μg doses of conjugate i.v. on day 0 into groups of five mice bearing measurable s.c. LNCaP tumors. At each antibody dose, the dose of radioactivity was about 5 μCi. On day 4 the mice were killed and dissected for biodistribution determinations.

The immune specificity of CYT-356-^{111}In tumor localization was determined by comparison of its biodistribution to that of CYT-103-^{111}In (an irrelevant antibody in this system) in LNCaP tumor-bearing and normal nude mice on day 4 after injection. Each conjugate was injected i.v. on day 0 into a group of five mice bearing measurable s.c. LNCaP tumors and into a group of three mice without tumors. On day 4, the mice were killed and dissected for pharmacokinetic determinations. Again, tumor-bearing mice were imaged immediately prior to dissection.

Similarly, CYT-356-^{111}In immune-specific tumor localization was assessed in nude mice bearing prostate tumors DU-145 and PC-3, which by immunohistological methods were shown not to express the antigen detected by CYT-356. Conjugate was injected i.v. on day 0 into groups of four tumor-bearing mice and on day 4 the mice were imaged and dissected.

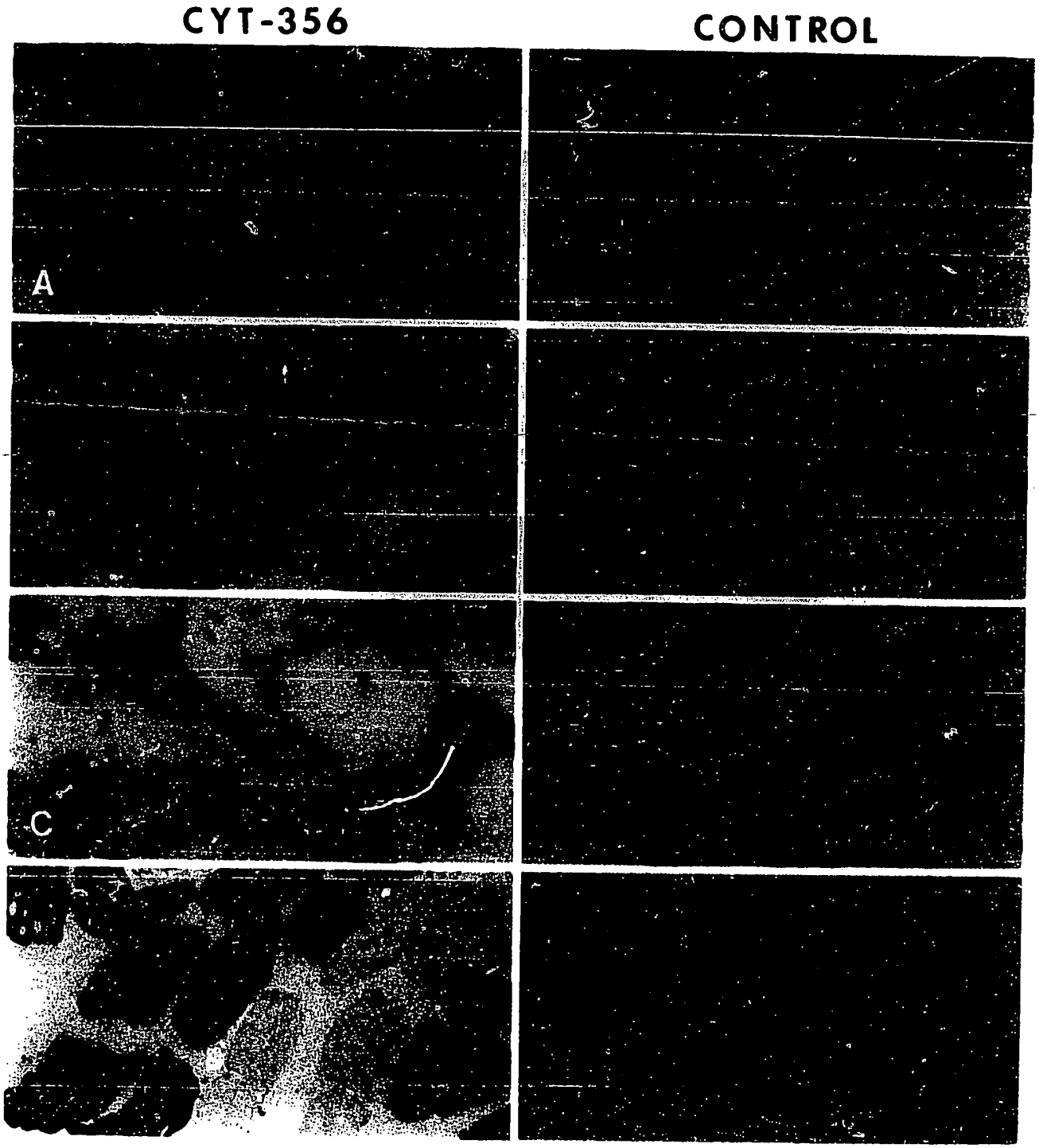

Fig. 1. Immunoperoxidase staining of CYT-356 on acetone-fixed frozen tissues. *A*, normal prostatic tissue; *B*, undifferentiated prostatic carcinoma; *C*, moderately differentiated prostatic carcinoma; *D*, skeletal muscle. Control sections were incubated with an irrelevant isotype-matched immunoconjugate (BL-3-GYK-DTPA). *Bar*, 25 μm.

Dissected tissues were weighed and the amount of ^{111}In in them was determined by gamma-counting. The data are presented as the ratio of the cpm/g in each organ to the cpm/g in blood (organ to blood ratio) and the percentage of ID/g in each organ. The biological half-life (whole body) of ^{111}In was calculated assuming a single exponential decay function from data obtained by placing the mice in a dose calibrator. Similarly, the blood half-life was calculated from the average percentage of ID/g in blood on days 1, 3, or 7.

RESULTS

Reactivity with Normal Human Tissues. The reactivity of both 7E11-C5 and CYT-356 with normal human tissues was assessed by immunoperoxidase staining of acetone-fixed frozen sections. In all sections, the staining seen with the irrelevant immunoconjugate BL-3-GYK-DTPA was used to define the background

Table 2 *Reactivity of CYT-356 on a ponel of human tumors*

Acetone-fixed frozen sections of human tumor tissue were stained by the ABC method with CYT-356 at 5 μg/ml.

Tumor type	Number positive/ number tested
Prostate carcinoma	10/10
Breast carcinoma	0/3
Colon carcinoma	0/3
Lung carcinoma	0/3
Renal cell carcinoma	0/3
Bladder carcinoma	0/3
Sarcoma	0/3
Melanoma	0/3
Lymphoma	0/3
Rhabdomyosarcoma	0/7
Leiomyosarcoma	0/3

staining. Table 1 shows the results of 7E11-C5 and CYT-356 staining on tissues from one or two individuals. In general, there was very little reactivity seen with normal human tissues. Neither 7E11-C5 nor CYT-356 stained liver, lymph node, adrenal gland, lung, gastrointestinal tissue, testis, thyroid, pancreas, spleen, or brain tissue. Both 7E11-C5 and CYT-356 were weakly reactive with both specimens of heart tissue, one of two specimens of kidney tissue (proximal tubules), and one of two specimens of skin (sweat glands). In sections of normal prostate tissue, epithelial cells were positively stained, glandular lumina were heterogeneously stained, and there was weak generalized staining of connective tissue with both antibody and immunoconjugate. Cytoplasmic staining of a subset of skeletal muscle cells (about 60%) was also evident in sections incubated with CYT-356 (Fig. 1*D*); these were identified as type 2 muscle fibers by ATPase staining. Mouse skeletal muscle showed an identical staining pattern (not shown). In all tissues examined, there was concordant staining by 7E11-C5 and CYT-356, indicating that, at this level of analysis, the immune specificity of the antibody has been preserved in the site-specific immunoconjugate.

Reactivity on a Panel of Human Tumors. A panel of three to seven specimens of each of 10 human tumor types and 10 specimens of pr state carcinoma were collected and stained with CYT-356. As shown in Table 2, CYT-356 reacted with all of the prostate carcinoma tumors tested but non of the sarcoma, rhabdomyosarcoma, leiomyosarcoma, melanoma, lymphoma, or breast, colon, lung, renal, or bladder carcinoma samples. Both undifferentiated (Fig. 1*B*) and moderately differentiated (Fig. 1*C*) prostate tumors stained more intensely than normal prostate (Fig. 1*A*).

Tumor Localization Kinetics. The biodistribution and imaging efficacy of CYT-356-^{111}In in both LNCaP tumor-bearing and normal nude mice was assessed on days 1, 3, and 7 after i.v. injection. The average dose injected into each mouse was 11.7 μg (~16 μCi by dose calibrator). Average tumor weights ± SE were 0.44 ± 0.11 g, 0.11 ± 0.03 g, and 0.33 ± 0.12 g for the groups dissected on days 1, 3, and 7, respectively. The average biological (whole body) half-life calculated from dose calibrator data, assuming a single exponential decay function, between day 0 and day 3 or between day 0 and day 7 was 11.7 ± 0.9 days or 8.4 ± 0.4 days, respectively. The half-life of the ^{111}In label in the blood calculated from the percentage of ID/g data for blood, assuming a single exponential decay, between days 1 and 3 or between days 1 and 7 was 2.3 days or 3.8 days, respectively. These values were calculated for the non-tumor-bearing mice only; the ability of the tumors to remove conjugate from the blood makes the calculation of these values inappropriate in tumor-bearing mice. These serum half-life values are similar to those measured for other site-specific murine IgG-GYK-DTPA-^{111}In conjugates but are less than the 6-8-day range reported for the serum half-life of murine IgG1 antibodies in mice (14).

Following i.v. administration, CYT-356-^{111}In localized to and imaged LNCaP xenografts. Localization of CYT-356-^{111}In to the LNCaP tumors increased from about 14% ID/g on day 1

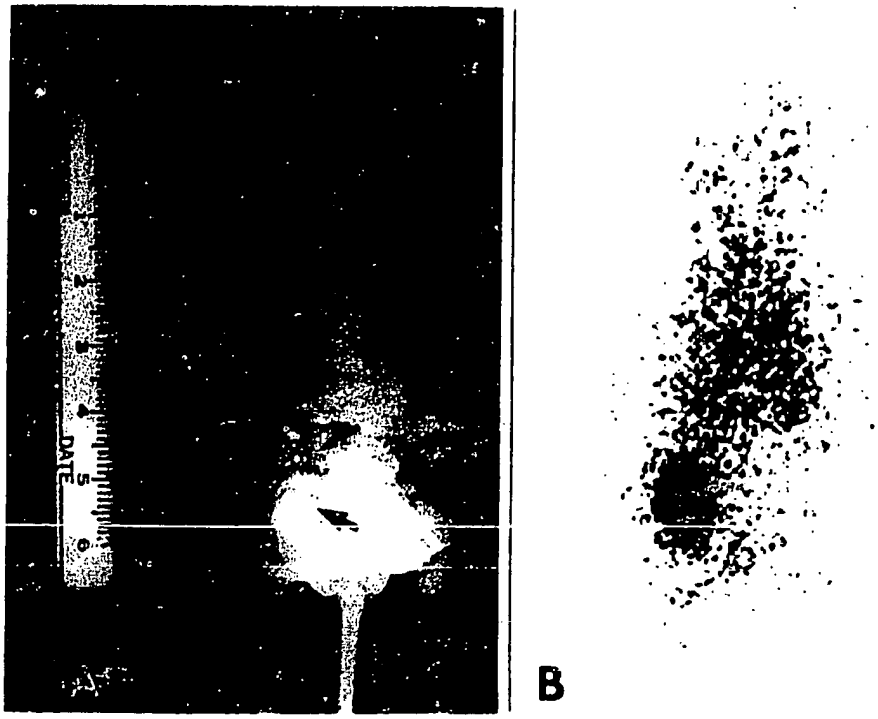

Fig. 2. Whole body scintigraphic image of a nude mouse bearing a s.c. LNCaP tumor. This image (*B*) was obtained 4 days after injection of 10 μg of CYT-356-^{111}In (specific activity = 1.5 μCi/μg). *Arrows* (*A*) indicate location of tumor.

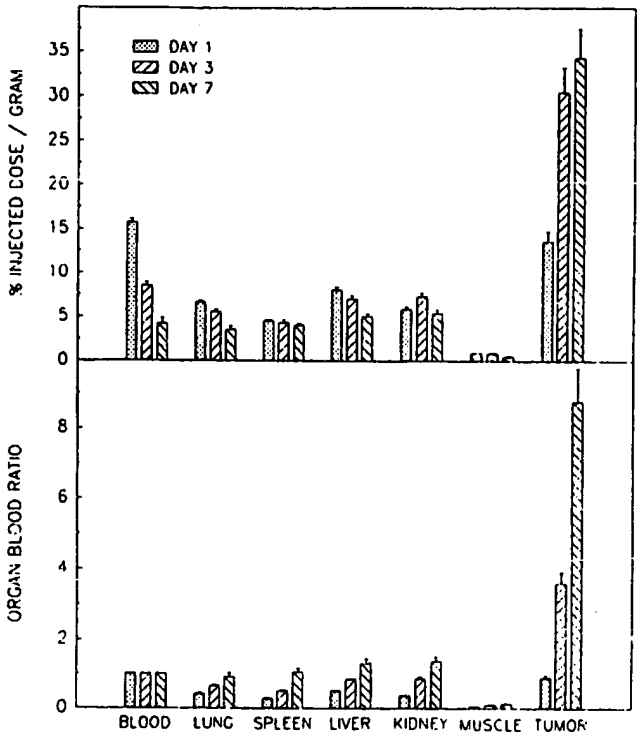

Fig. 3. Biodistribution of CYT-356-^{111}In in nude mice bearing LNCaP xenografts at 1 day (□), 3 days (▨), and 7 days (▩) after i.v. injection of 10 μg of immunoconjugate (specific activity = 1.5 μCi/μg). The percentage of ID/g and organ to blood ratios were calculated as described in "Materials and Methods."

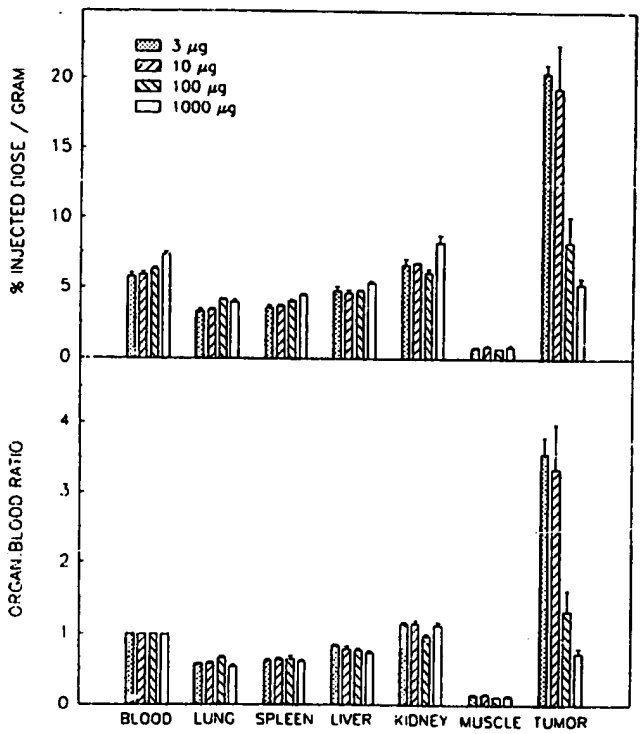

Fig. 4. Effect of the amount of immunoconjugate administered on the biodistribution of CYT-356-^{111}In. LNCaP xenograft-bearing nude mice were given injections of 3 (□), 10 (▨), 100 (▩), or 1000 (□) μg of CYT-356-^{111}In on day 0. The specific activity of the labeled immunoconjugate was varied so that 5 μCi of radioisotope were injected at each immunoconjugate dose. Mice were dissected on day 4 and the percentage of ID/g and organ to blood ratios were calculated as described in "Materials and Methods."

to about 30% ID/g on days 3 and 7 (see Fig. 3). Collectively, these data show that CYT-356-^{111}In localizes to LNCaP xenografts rapidly, reaching maximum levels by 2 or 3 days after injection. As shown in Fig. 2, tumor images were clearly discernible by day 4. This is consistent with the average tumor to blood ratios of 3.6:1 and 8.8:1 on days 3 and 7, respectively (Fig. 3).

No unusual localization was seen to any nontumor tissue or organ of tumor-bearing mice (Fig. 3) or normal mice (data not shown). Importantly, CYT-356 did not localize to skeletal muscle, even though it exhibited *in vitro* reactivity with murine skeletal muscle similar to that seen with human muscle. The level of radioactivity in all normal tissues and organs examined was at or below that seen in the blood (Fig. 3).

Dose Response of Tumor Localization. The relationship between antibody dose and tumor uptake was assessed by giving injections of varying amounts (3, 10, 100, and 1000 μg) of CYT-356-^{111}In to groups of tumor-bearing mice. In this experiment, LNCaP tumors weighed an average of 0.15 g on the day of dissection. The tumor to blood ratio and the percentage of ID/g of CYT-356-^{111}In localizing to LNCaP xenografts 4 days after injection were highest at the smallest dose of immunoconjugate (3 μg; Fig. 4). The values were similar at the 10-μg dose but substantially lower at the 100-μg dose. These data suggest that saturation of antigenic sites in the tumors occurred at a dose of immunoconjugate between 10 and 100 μg.

Immune Specificity of Tumor Localization. The immune specificity of CYT-356-^{111}In tumor localization was determined by comparing its biodistribution to that of CYT-103-^{111}In (an irrelevant antibody in this system) in LNCaP tumor-bearing and normal nude mice on day 4 after injection. In this experiment, CYT-356-^{111}In localized to LNCaP tumors but the irrelevant conjugate did not (Fig. 5). Uptake in normal organs of the mice was the same for either conjugate. Furthermore, CYT-356-^{111}In did not localize to the antigen-negative prostate tumors PC-3 and DU-145 (Table 3). Thus, tumor localization by CYT-356-^{111}In was tumor antigen specific.

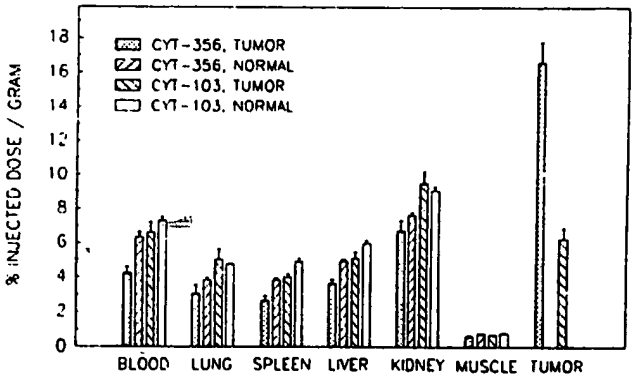

Fig. 5. Specificity of CYT-356-^{111}In tumor localization. Normal and LNCaP xenograft-bearing mice were given injections of either CYT-356-^{111}In (10 μg; specific activity = 4.4 μCi/μg) or CYT-103-^{111}In (10 μg; specific activity = 5.7 μCi/μg) on day 0. Mice were dissected on day 4 and the percentage of ID/g was calculated for tumor-bearing mice treated with CYT-356 (□), normal mice treated with CYT-356 (□), tumor-bearing mice treated with CYT-103 (▩), and normal mice treated with CYT-103 (□).

DISCUSSION

This study has demonstrated that CYT-356, a site-specifically labeled immunoconjugate of the prostate carcinoma-associated

Table 3 *Distribution of CYT-356-^{111}In in nude mice bearing antigen-negative prostate tumors DU-145 and PC-3*

Tumor-bearing nude mice received ~10 μg CYT-356-^{111}In i.v. on day 0 and were imaged and dissected on day 4.

Organ	Tumor type (%ID/g)[a]	
	DU-145	PC-3
Blood	7.1	7.5
Lung	3.8	4.0
Spleen	4.5	5.0
Liver	6.1	5.9
Kidney	4.9	5.0
Tumor	5.1	5.1
Muscle	0.7	0.6

[a] Mean percentage of ID/g; $n = 3$ (DU-145) or $n = 2$ (PC-3).

monoclonal antibody 7E11-C5, localizes well to and images prostate tumor xenografts. Further, CYT-356 exhibited little reactivity with normal human tissues. Collectively, these data demonstrate that CYT-356-^{111}In may be clinically useful for radioimmunoscintigraphy of prostate cancer.

Immunohistological staining of frozen tissues showed strong staining of all prostate adenocarcinomas tested. No reactivity was seen with a panel of 10 other human tumor types. Thus, in contrast to most other prostate tumor-reactive monoclonal antibodies, CYT-356 selectively reacts with prostate adenocarcinomas.

Neither native antibody 7E11-C5 nor 7E11-C5-GYK-DTPA immunoconjugate (CYT-356) reacted with the majority of normal human tissues tested, concordant with the data of Horoszewicz *et al.* (8). However, we observed normal skeletal muscle reactivity, which was not previously described (8). This discrepancy may be related to differences in the tissue fixation methodology used, because we worked only with acetone-fixed frozen tissue while Horoszewicz *et al.* (8) used formalin fixation. This reactivity is probably of little consequence to the *in vivo* localization of the immunoconjugate, because immunoperoxidase staining of murine skeletal muscle demonstrated this same reactivity, yet the conjugate did not localize to skeletal muscle of the mice in the imaging studies. Furthermore, the amount of ^{111}In in all normal organs, including muscle, was the same in mice which received either CYT-356 or the irrelevant immunoconjugate CYT-103. Finally, additional experiments in cynomolgus monkeys failed to reveal abnormal uptake of CYT-356-^{111}In into muscle in this non-human primate.[3]

Although normal prostate tissues were weakly positive, the consequence of this reactivity on *in vivo* localization and imaging is not known, because the animal model we employed had no reactive normal prostate tissue component. However, the utility of CYT-356 should not be diminished even if it does localize to normal prostate in men, because the primary application for this immunoconjugate will be to diagnose the occurrence and extent of metastatic spread. Such metastases would be revealed as areas of radioactivity outside the prostate. Weak staining of kidney and connective tissue was also observed, the significance of which is as yet unknown. However, such slight reactivity has been observed with many different monoclonal antibodies[4] and, therefore, does not appear to be an antigen-dependent phenomenon.

The biochemical nature of the antigen recognized by 7E11-C5 is the subject of active investigation but is, as yet, ill defined. Preliminary results indicate that this antigen is a M_r 100,000 molecule distinct from other reported prostate organ-associated antigens such as pr static acid phosphatase and prostate-specific antigen (15). Furthermore, immunofluorescence studies have dem nstrated that 7E11-C5 binds to the cell surface of about 20% of live LNCaP cells but is also found in the cytoplasm of the majority of acetone-fixed cells (data not shown). This membrane reactivity may be due to authentic membrane antigen expression or, alternatively, may result from membrane adsorption of antigen from the extracellular milieu (8). The relative contribution of membrane and intracellular antigen to the tumor localization of CYT-356 is difficult to assess, because monoclonal antibodies against both intracellular (16, 17) and cell surface/extracellular (10, 18, 19) antigens have been shown to localize to tumor xenografts. Clearly, the human tumor xenograft localization data indicate that at least a portion of the immunohistologically detected 7E11-C5 antigen is accessible to antibody binding *in vivo*. In fact, tumor uptake of CYT-356-^{111}In (up to 30% ID/g) is similar to that of other antitumor antibodies including B72.3 (10, 18), anti-carcinoembryonic antigen (19), and the antiprostate antibody TURP-27 (20). Furthermore, the tumor uptake induced by CYT-356-^{111}In was antigen dependent, because an irrelevant immunoconjugate (CYT-103-^{111}In) did not localize to or image these xenografts, nor did CYT-356-^{111}In localize to or image xenografts of two other transplantable human prostate tumor lines (PC-3 and DU-145) that do not express the CYT-356 antigen.

In the LNCaP xenograft model, a dose of CYT-356-^{111}In between 10 and 100 μg appeared to saturate medium-sized (150 mg) tumors. Scaled to human doses, these would represent 30 and 300 mg, which are relatively high doses for imaging applications. In the xenograft model system, the tumor to blo d ratios decreased at higher doses because of the large amount f conjugate that remained in the circulation. While the significance of this finding to the clinical utility of the immunoc n-jugate is not clear, these data suggest that tumor uptake and image quality may begin to decrease as the total antibody dose increases.

While murine xenograft results are not entirely predictive of clinical activity in humans, the results presented here suggest that CYT-356 has potential utility in the diagnosis and therapy of prostate cancer. Clinical trials designed to assess the safety and efficacy of CYT-356-^{111}In for radioimaging of primary and metastatic prostate carcinoma lesions are in progress.

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